

ScreenTape® P200 monitors GST-Tag cleavage from purified GST fusion proteins

ScreenTape P200 allows rapid and detailed analysis of GST-Tag removal from GST fusion proteins between 10 to 200kDa. P200 helps you monitor the efficiency of the cleavage reaction and optimise this enzymatic reaction. In a single analysis step, P200 will accurately size your purified protein before and after tag removal, whilst measuring its purity and quantity, therefore effectively streamlining your recombinant protein purification protocol.

Tape Used – ScreenTape P200

Time to result – Less than one minute per sample

Sample volume per test – 2µl

Specifications

Sizing range: 10 to 200kDa

Molecular weight accuracy (average deviation) <10%

Molecular weight precision ≤2% CV

Suggested sample conc.: 100 to 1000µg/ml

Typical sensitivity: 5µg/ml

Quantitative range: 5 to 5000µg/ml

Analysis Results

Automated peak location and annotation

Automated protein sizing, purity and quantity

GLP standard report and print-out

Introduction

Glutathione S Transferase (GST) is an epitope tag that is commonly engineered onto recombinant proteins for expression and purification applications. Glutathione transferases bind glutathione with high affinity and specificity, allowing glutathione based affinity resins to successfully purify GST-Tagged proteins. GST is a 24kDa protein making it much larger than most epitope tags and as a result, more prone to degradation. During the purification process of GST fusion proteins, collected fractions are routinely checked for protein content using traditional methods such as SDS-PAGE analysis. ScreenTape P200 demonstrates accurate and reproducible performance for automated analysis of on-column cleaved protein fractions and GST-Tag elution products. The fully integrated analysis includes peak annotation, accurate molecular weight determination and percent purity values, making P200 a method of choice for GST-Tag applications.

Materials and Methods

To illustrate how ScreenTape P200 can analyse GST-Tagged proteins during cleavage, samples were generated on a glutathione sepharose matrix in a Zeba micro-spin column (Pierce) using PreScission™ protease reagents and methods (GE Healthcare). A GST-Tagged protein of 130kDa was incubated at 0.4µg/µl overnight at 8°C in PreScission protease cleavage buffer containing 0.008U/µl PreScission protease (see Figure 1). The digested product, which now contained the protein devoid of its GST-Tag, PreScission protease and free GST, was incubated with glutathione sepharose 4B for 30 minutes on ice before loading onto the Zeba micro-spin column. The protease and free GST were immobilised onto the glutathione matrix, leaving the protein of interest free in solution. Following centrifugation and two wash and spin steps with glutathione free buffer, all the protein was collected into three fractions. Two wash and spin steps in elution buffer containing 20mM reduced glutathione were used to regenerate the column, resulting in two fractions containing the eluted GST and protease. An aliquot from each of the collected fractions was analysed on ScreenTape P200.

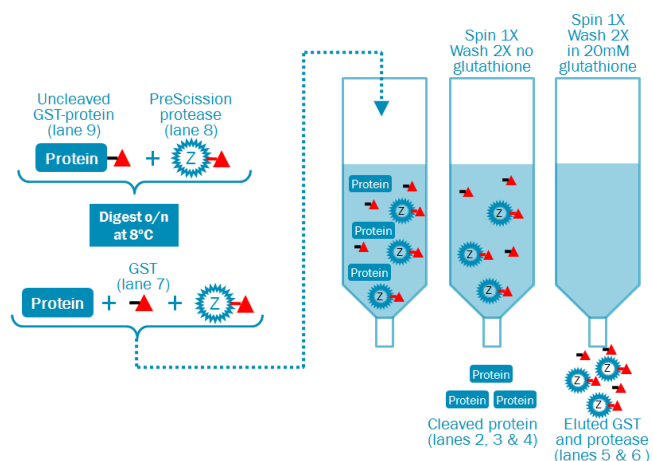


Figure 1: Method using PreScission reagents to cleave GST-Tag from a GST fusion protein. The GST-Tag and the protease have a high affinity for the glutathione matrix, allowing cleaved proteins to be segregated from the enzyme and GST. Aliquots of samples that were collected during the purification procedure are described in the diagram and associated with their corresponding lane positions during analysis on ScreenTape P200.

ScreenTape P200 Analysis Procedure

All samples were prepared for ScreenTape P200 according to the Lab901 protocol, which includes sample pre-staining, therefore avoiding lengthy staining and destaining procedures that are common to SDS-PAGE methods. Tubes containing prepared protein samples were placed in the TapeStation® with ScreenTape P200 and tips. After clicking “START” on the software driven menu, full analysis of the samples was achieved and archived, with no user intervention, within a minute per sample.

Results

The ScreenTape platform displays fully analysed results in less than one minute per sample (shown in Figure 2, left panel), which includes protein molecular weights and % purity values that are automatically calculated and presented in a gel image, electropherogram and table. Peaks are automatically annotated allowing you to check for fusion protein tag removal easily (the transition shows a 24kDa shift from around 167kDa to 143kDa) and to monitor column regeneration after glutathione elution. By using the integrated Lab901-GeneTools software profile overlay function, it was possible to fully appreciate the molecular weight shift in the target protein before and after GST-Tag removal (Figure 2, screen-grab in the right panel). Furthermore, the P200 results were archived automatically and printed to a GLP compliant report with a single mouse click.

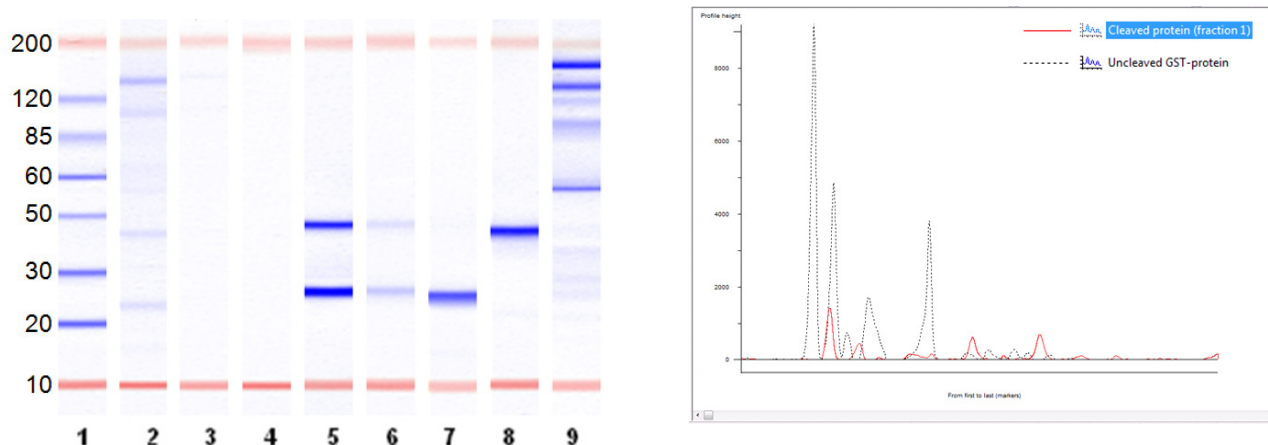


Figure 2: Analysis of GST cleavage from a fusion protein of 167kDa on ScreenTape P200. In the left panel: Lane 1 contains a molecular weight standard (band sizes in kDa). Lanes 2 to 9 contain results for the samples defined in Figure 1. GST-Tag removal can be confirmed by comparing the molecular weight of the major protein in lane 2, (143kDa) with that of the major protein in lane 9 (167kDa). This corresponds to the removal of GST seen alone in lane 7 (24kDa) and also present in the glutathione eluant in lanes 5 & 6. The protease (46kDa) is observed in lanes 5 and 6, and can be seen alone in Lane 8. The right panel shows the screen-grab of a profile overlay of lanes 2 and 9 showing the target protein before (dotted black) and after GST-Tag cleavage (red). The protein preparation also contains several other contaminating proteins, which are easily monitored on the P200 platform.

Benefits of using ScreenTape P200 for monitoring GST-Tag removal:

- **Accelerate your purification workflow** - P200 is a rapid and straightforward method for monitoring the cleavage of a target protein from its GST-Tag. By automating electrophoresis analysis, GST purification methods are streamlined, allowing you to proceed faster with downstream applications.
- **Have greater confidence in your results** - Electropherogram overlays, made possible by the Lab901-GeneTools software, allow you to verify complete GST-Tag cleavage.
- **Limit sample preparation steps** - No buffer exchange is needed prior to analysis as P200 is fully compatible with the protease and glutathione buffers found in GST purification protocols.
- **Use a safer electrophoresis method** - Reagents are pre-packaged making P200 a convenient, hands-free and safe electrophoresis method.
- **Improve accuracy and reproducibility** - Through QC and automation, P200 delivers more accurate and reproducible electrophoresis results than traditional SDS-PAGE, therefore increasing confidence in GST-Tag cleavage and purification yield calculation.
- **Deliver GLP compliant results** - Results are seamlessly archived in a GLP compliant format, which allows different batches to be compared and protein manufacturing to keep accurate records.

For a full list of application notes covering additional protein purification analysis methods, please visit www.lab901.com.

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